OBITUARY

A tribute to Ko Shimamoto (1949–2013)

Paula Suárez López1,*, Hiroyuki Tsuji2 and George Coupland3

1 Centre for Research in Agricultural Genomics, CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain
2 Laboratory of Plant Molecular Genetics, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan
3 Max Planck Institute for Plant Breeding Research, Carl von Linné Weg 10, D-50829 Cologne, Germany

* To whom correspondence should be addressed. E-mail: paula.suarez@cragenomica.es

Received 7 February 2014; Revised 7 February 2014; Accepted 12 February 2014

Professor Ko Shimamoto tragically passed away on 28 September 2013, at the age of 63, after an illness. He was born in October 1949 in Wakayama Prefecture, a warm southern area near Nara where he ultimately established his laboratory. He grew up in Toyohashi city near Nagoya, in the central part of Japan. ‘Because Ko and I shared the same hometown’ — evokes Hiroyuki Tsuji — ‘we sometimes enjoyed talking about changes in the architecture in these cities’.

Ko graduated from the Department of Agriculture at Kyoto University and then moved to the USA to become a graduate student at the University of Wisconsin-Madison in 1974. After receiving his PhD degree in genetics in 1980, he started postdoctoral training at the Friedrich Miescher Institute, Basel, Switzerland. In 1984, he returned to Japan to join the Plantech Research Institute, which was established by the Mitsubishi Chemical Corporation, initiating a programme using biotechnology in rice breeding. He immediately began experimenting with conditions for rice transformation, and in 1989 he succeeded in regenerating the first fertile transgenic rice plants from protoplasts (Shimamoto et al., 1989). This achievement resulted from his highly sophisticated tissue culture techniques that were continuously perfected during his research career. He generated several new cultivars of rice by means of protoplast culture and regeneration, putting his research group at the forefront of rice breeding. In 1993, he moved to the newly established Nara Institute of Science and Technology and started his own new laboratory in the Graduate School of Biological Sciences as a Professor of Plant Molecular Genetics. He launched three different themes in the new laboratory, plant innate immunity, gene silencing, and flowering, and each was full of exciting achievements. With these choices, Ko devoted his scientific life to understand traits with enormous relevance for agriculture and to develop tools that could be useful for rice improvement.

Plant innate immunity

Ko’s laboratory shed light on the mechanisms of plant immunity in rice. When pathogens infect plants, pathogen-derived molecules are recognized by pattern recognition receptors at the cell surface, triggering a signalling cascade that leads to protection against the pathogen (reviewed by Kawano and Shimamoto, 2013). An early response is the production of reactive oxygen species (ROS), which can be toxic for pathogens and also induce plant cell death at the site of infection, an effect known
as the hypersensitive response (reviewed by Senthil-Kumar and Mysore, 2013). At the turn of the century, Ko’s group described that the small GTPase Rac1 enhances resistance to two pathogens, rice blast fungus and bacterial blight. Through activation of NADPH oxidase, Rac1 increases the production of ROS and regulates cell death in rice (Kawasaki et al., 1999; Ono et al., 2001). Since then, his laboratory has made remarkable contributions to the dissection of this defence pathway, identifying numerous Rac1-interacting proteins as well as upstream regulators and downstream targets.

Ko’s group characterized at least three upstream regulators of Rac1 in the response to fungal pathogens: a subunit of the heterotrimeric G protein (Dwarf1), which is an important signal transduction complex in animals and plants (Suharsono et al., 2002); serotonin, a well-known animal neurotransmitter, which plays a role in plant defence in a process mediated by Rac1 and Dwarf1 (Fujiwara et al., 2010); and Pit, a nucleotide-binding domain and leucine-rich repeat-containing protein that affects ROS production and the hypersensitive response (Kawano et al., 2010).

Step by step, he described interactions of Rac1 with other proteins involved in plant immunity. These Rac1-interacting factors include proteins with a range of functions, such as kinases, receptor kinases, two guanine nucleotide exchange factors that activate Rac1, chaerones and co-chaperones, a transcription factor, and a receptor for protein kinase C that in animals acts as a scaffolding protein with important roles in development, ageing, cancer, and various signalling processes (Akamatsu et al., 2013; Chen et al., 2010; Lieberherr et al., 2005; Nakashima et al., 2008; Thao et al., 2007; Wamaitha et al., 2012; Yamaguchi et al., 2012; Yamaguchi et al., 2013). These interactions, detected by diverse methods, were confirmed through proteomic analyses, which identified additional Rac1-interacting proteins (Fujiwara et al., 2009; Fujiwara et al., 2006; Tsunezuka et al., 2005). The work of his group led to the functional characterization of many of these Rac1 interactors, establishing their specific roles in defence signalling. Altogether, these results indicate the formation of protein complexes regulating innate immunity in rice.

During the last few years, the work of Ko’s laboratory showed that a membrane-bound chitin receptor (CERK1) triggers a signal transduction cascade in response to pathogen-derived chitin. This cascade rapidly activates Rac1 and induces defence responses. CERK1 binds several Rac1-interacting proteins in the endoplasmic reticulum and this complex seems to traffic from the endoplasmic reticulum to the plasma membrane, where the complex interacts with Rac1 (Akamatsu et al., 2013; Chen et al., 2010; Yamaguchi et al., 2013). The work of Akamatsu et al. (2013) includes an illustrative model of the major findings of Ko’s group in the field of plant immunity.

Downstream effectors of Rac1 were also identified by Ko’s laboratory. Rac1 downregulates a metallothionein, MT2b, which acts as an ROS scavenger (Wong et al., 2004). Together with the role of Rac1 on NADPH regulation, this suggests that Rac1 induces ROS production and suppresses ROS scavenging, making a double contribution to the oxidative burst associated with disease resistance. Later work of Ko’s group showed that the activation of ROS production results from direct interaction between Rac1 and the catalytic subunit of NADPH oxidase (Rboh) at the plasma membrane (Wong et al., 2007). Rac1 also activates an enzyme involved in lignin biosynthesis, leading to lignin accumulation (Kawasaki et al., 2006). This also contributes to plant defence, as lignin acts as a physical barrier against pathogens. A basic helix-loop-helix transcription factor, RAI1 (Rac Immunity1), is another Rac1-downstream factor, which is phosphorylated by kinases present in the Rac1 complex and increases resistance to blast fungus (Kim et al., 2012).

His last published work is a collaboration with the laboratory of Chojiro Kojima reporting the crystallization of Rac1 and a preliminary X-ray crystallographic analysis to determine Rac1 structure (Kosami et al., 2014).

Therefore, Ko made significant advances in the molecular mechanisms underlying the response of rice to pathogens. He also helped to understand the roles that proteins with well-known functions in animals play in plants.

Flowering

Ko also made major contributions to understanding the molecular control of photoperiodic flowering. His early work related to understanding the short-day flowering response in rice, and then later broadened to study the role of FT-like proteins as systemic signals in diverse photoperiodic responses. His first important contribution was the demonstration that the photoperiodic flowering pathways of Arabidopsis and rice involve similar components functioning in the same order, but which confer the opposite response to day length (Hayama et al., 2003). Arabidopsis is a long-day plant and is therefore induced to flower under long days, whereas these conditions repress flowering in rice. At this time, CONSTANS (CO) transcription factor had been shown to have a central role in the photoperiodic response of Arabidopsis by activating transcription of FLOWERING LOCUS T (FT) in response to long days, and CO transcription had been shown to be regulated by GIGANTEA (GI). Furthermore, the CO homologue HEADING DATE 1 (Hd1) and the FT homologue HEADING DATE 3a (Hd3a) had been identified in rice as quantitative trait loci contributing to photoperiodic flowering. However, how these proteins could confer long-day flowering in Arabidopsis but short-day flowering in rice was unclear. Ko’s group showed that overexpression of the rice homologue of GI (OsGI) in transgenic rice plants caused increased expression of Hd1, but that in contrast to Arabidopsis this was associated with late flowering and reduced Hd3a expression. Transient expression assays demonstrated that Hd1 acted as a transcriptional repressor of Hd3a. Thus, they proposed that the photoperiodic response of rice involves transcriptional repression of Hd3a by Hd1 under long days, but that this does not occur under short days, so flowering proceeds under these conditions. This influential model suggested that the core photoperiodic flowering pathway involving GI–CO–FT is highly conserved among flowering plants, but that changes in the connectivity between components are responsible for evolution of photoperiodic responses. More recently, many more rice flowering-time genes have been identified adding...
complexity, but the connections identified by Ko’s group remain central to these models.

The work of Hayama et al. (2003) demonstrated that activation of *Hd3a* transcription under short days was the photoperiodic output that led to early flowering. This result stimulated Ko to study more thoroughly the role of FT-like proteins in flowering of rice, and his group showed that Hd3a and the related protein RICE FLOWERING LOCUS T1 (RFT1) are essential for rice flowering, because the double mutant *hd3a rft* does not flower under long or short days (Komiya et al., 2008). This result contrasts with *Arabidopsis*, where double mutants of *FT* and its homologue TWIN SISTER OF FT are day-length insensitive, but still able to flower. The idea that Hd3a and RFT are so closely associated with flowering in rice inspired Ko to focus his group on understanding their function in flowering-time control. By this time, it had been shown in *Arabidopsis* that FT promotes flowering when transcribed in the leaf vasculature, but that FT protein acts at the shoot apical meristem by interacting with the bZIP transcription factor FLOWERING LOCUS D (FD), and that the floral promotive activity of FT-like genes can cross graft junctions in *Arabidopsis* and tomato. As reviewed in detail elsewhere (Kobayashi and Weigel, 2007; Turck et al., 2008), there was speculation based on these observations that a product of FT might represent the graft transmissible substance florigen, the long-sought after signal made in the leaves that induces flower development at the shoot apex. Ko’s group aimed to test directly whether Hd3a protein was able to move from the leaves to the meristem. These results suggested that Hd3a protein could be translocated to the nucleus where it interacts with OsFD1. This complex can then bind to DNA and recognizes a motif in the promoter of the *OsMADS15* gene that encodes a MADS box transcription factor closely related to APETALA1, which confers floral meristem identity in *Arabidopsis* (Taoka et al., 2011). The 14-3-3 protein was therefore defined as functioning as an intracellular receptor for Hd3a. Because Ko was enthusiastic to incorporate novel techniques in his lab, he combined novel cell-biology techniques, structural biology, biochemistry, and molecular genetics to reveal the protein complex containing Hd3a. These highly influential experiments provide a structural and mechanistic basis for the action of FT-like proteins and demonstrate how they act to regulate gene expression.

Thus, over the 8 years between the appearance of the Hayama et al. (2003) and Taoka et al. (2011) papers, the work of Ko and his collaborators dramatically changed our perception of photoperiodic flowering. They helped provide a genetic and mechanistic framework for understanding short-day responses in rice, but more radically identified FT-like proteins as mobile signals and provided a structural understanding of how they act to regulate gene expression. These major contributions provide a basis for future groups to follow Ko’s lead and provide an understanding of how the changes in gene expression induced by FT-like proteins change the developmental identity of organs.

Ko was a good example that brilliant scientists excel also at attracting great talents to their labs. Among the members who made important contributions to the progression of the flowering field in the Shimamoto laboratory, we have to mention Shuji Yokoi, Shojiro Tamaki, Takeshi Izawa, Ryo Ishikawa, Reina Komiya, Ryosuke Hayama, and Yasuyuki Takahashi.

### Other contributions

Other developmental and physiological responses are controlled by photoperiod in addition to flowering. These include tuberization of potato and bud dormancy of trees. Ko’s group collaborated with that of Salomé Prat to examine whether FT-like proteins control tuberization in potato (Navarro et al., 2011). Normally, potato tubers are formed in response to short days but they showed that transgenic potato carrying the *RoiC:Hd3A* transgene formed tubers under non-inductive long days. This observation suggested that FT-like proteins do control this photoperiodic response from the leaves. Consistent with this idea, they identified the potato FT-like gene *StSP6a* as being transcriptionally induced in response to short days in leaves and stolons and to cause constitutive
we met at a conference, Ko greeted me with a cheerful smile and we discussed openly the latest results from his laboratory, as well as my results. Speaking with him was always one of the most enjoyable and encouraging moments in scientific conferences.

Ko enjoyed showing his guests around the ancient city of Nara, with its beautiful historic wooden buildings. ‘I had the pleasure to meet Ko at many international meetings, to host him in Cologne and to be hosted twice by him in Nara’ remembers George Coupland. ‘He was always an entertaining, motivated, and friendly companion. In Nara, he was a generous host, and I remember him taking a small group of us one November to see the autumn colours of maple trees spectacularly illuminated after dark’.

Ko’s death has deeply saddened his colleagues, friends, former and present students, and also the scientific community. We have lost him far too early, and while he was still very actively contributing to our field. He will be remembered as an innovator in plant molecular genetics. We are grateful for the opportunity to experience his enthusiasm for plant science and will remember him with loving memories.

Shimamoto-san, Arigato

References


Kawano Y, Shimamoto K. 2006. Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an...


